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# Clinical Evaluation of Blood Pressure Lowering, Endothelial Function Improving, Hypolipidemic and Anti-Inflammatory Effects of Pomegranate Juice in Hypertensive Subjects

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**Pomegranate (*Punica granatum* L.) juice (PJ) contains different types of antioxidants and bioactive polyphenols and has been reported to promote cardiovascular health through several mechanisms. The present study aimed to examine the effects of 2-week intake of fresh PJ on blood pressure, flow-mediated dilatation (FMD), serum lipid profile and concentrations of inflammatory and endothelial function biomarkers. Twenty-one hypertensive patients (aged 30–67 years) were recruited into the trial and assigned to receive either PJ (150 ml/day in a single occasion between lunch and dinner; $n = 11$) or the same amount of water ($n = 10$) for a period of 2 weeks. Systolic (SBP) and diastolic (DBP) pressures together with FMD and serum concentrations of lipid profile parameters, apolipoproteins A and B, intracellular adhesion molecule-1 (ICAM-1), vascular endothelial adhesion molecule 1 (VCAM-1), E-selectin, high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6) were measured at baseline and at the end of trial. PJ consumption was associated with significant reductions in SBP ($p = 0.002$) and DBP ($p = 0.038$) but not FMD ($p > 0.05$). Serum levels of VCAM-1 ($p = 0.008$) were significantly reduced by PJ while those of E-selectin were elevated ($p = 0.039$). However, no significant effect was observed from PJ on serum levels of ICAM-1, hs-CRP, lipid profile parameters, apolipoproteins and IL-6 in any of the study groups ($p > 0.05$). Consumption of PJ for 2 weeks has effective hypotensive effects, and may improve endothelial function by decreasing serum concentrations of VCAM-1. These findings suggest PJ as a beneficial cardioprotective supplement for hypertensive subjects. Copyright © 2013 John Wiley & Sons, Ltd.**

*Keywords:* blood pressure; atherosclerosis; alternative medicine; pro-inflammatory cytokines.

## INTRODUCTION

Cardiovascular disease (CVD) encompasses a wide range of abnormalities that initiate from atherosclerosis and progresses over time into ischemic heart disease, cardiomyopathy and acute coronary syndrome. CVD is often accompanied by an increased production of free radicals, most notably reactive oxygen species (ROS) such as superoxide and hydroxyl radicals (Das and Maulik, 1995). Increased generation of ROS in vascular cells can interfere with normal anti-coagulation and anti-inflammatory properties and induce several pathologic abnormalities in the endothelium (Cai and Harrison, 2000). In light of the extensive research in the past two decades, the classical view toward endothelium has been changed drastically. Endothelium is no more considered as a simple physical barrier between intravascular and interstitial compartments, but a large and active organ with endocrine functions as well as a pivotal role in maintaining vascular haemodynamics and homeostasis (Marx and Grant, 2007).

Upon endothelium dysfunction, increased vascular permeability allows plasma components including low-density lipoprotein (LDL) to penetrate and deposit in the subendothelial space. Thus, endothelial dysfunction is considered to be the first triggering step in the atherogenesis process (Bonetti *et al.*, 2003; Weissberg, 1999). Impaired endothelial function is characterized by a reduction in the bioavailability of nitric oxide (NO), which is a potent vasodilator with anti-inflammatory and anti-proliferative properties, and inhibitory activity against platelet adhesion and aggregation (Deanfield *et al.*, 2007; Furchgott and Zawadzki, 1980). Another characteristic of endothelial dysfunction is overexpression of cell adhesion molecules (CAMs), followed by their release into the circulation. CAMs possess inflammatory properties and mediate the marginalization, rolling and tethering of leukocytes along the endothelium, thus playing a pivotal role in the development and progression of atherosclerosis (Ballantyne and Abe, 1997; Cybulsky *et al.*, 1999; Galkina and Ley, 2007). Endothelial function is measured *in vivo* through measuring flow-mediated dilation (FMD) in the brachial artery. FMD has been

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proven to be a strong predictor of cardiovascular events (Gokce *et al*., 2002; Rossi *et al*., 2008; Widlansky *et al*., 2003; Yeboah *et al*., 2007).

There is a strong pile of evidence indicating diverse biological activities and health benefits of plant phytochemicals, in particular phenolics and flavonoids (Hodgson and Croft, 2010; Sahebkar, 2010a, b, Hu *et al*., 2011; Xiao *et al*., 2011; Huang *et al*., 2012; Ikemura *et al*., 2012; Mohammadi *et al*., 2012; Panahi *et al*., 2012a, b; Sahebkar, 2012a, b; Shin *et al*., 2012). Large-scale prospective cohorts have suggested an inverse association between consumption of fruits and vegetables rich in flavonoids and CVD mortality (Liu *et al*., 2000; Mink *et al*., 2007). Furthermore, an increasing number of epidemiological studies have consistently shown a protective effect of foods rich in polyphenols (fruit, tea, wine, and cocoa or chocolate and specially citrus fruit) against several CVD risk factors such as LDL oxidation, hypertension and endothelial dysfunction (Hamdy *et al*., 2003; Keogh *et al*., 2005; Wu and Meininger, 2002).

*Punica granatum* L. (Punicaceae) fruit (Pomegranate) is considered as a heart-healthy fruit juice (Basu and Penugonda, 2009). Pomegranate is rich in polyphenolic type antioxidants including tannins, anthocyanins (de Nigris *et al*., 2007) and several other types of flavonoids (Sudheesh *et al*., 1997). The soluble polyphenol content in pomegranate juice (PJ) normally ranges between 0.2 and 1.0%, depending on the variety, and mainly comprises tannins, ellagic anthocyanins, catechins, and gallic and ellagic acids (Ben Nasr *et al*., 1996). In spite of the previously reported cardiovascular health benefits of pomegranate and its rich content of polyphenols and flavonoids (Anonymous, 2007; Basu and Penugonda, 2009; Fuhrman and Aviram, 2007; Ross, 2009; Stowe, 2011), there have been very few studies on the anti-hypertensive effects of PJ in clinical setting. In addition, the impact of PJ on endothelial function as well as circulating levels of CAMs – as important mediators in endothelial dysfunction and pathogenesis of atherosclerosis – has remained deeply unexplored, and its rich content of polyphenols and flavonoids led to the hypothesis being tested in the present study, whether supplementation with PJ could ameliorate endothelial function and lower blood pressure in hypertensive subjects.

## SUBJECTS AND METHODS

**Subjects.** Twenty-one hypertensive patients aged 30–67 years were recruited for this trial. Patients were selected from those referred to the Hypertension Clinic at the Isfahan Cardiovascular Research Institute. The Ethics Committee at the Isfahan University of Medical Sciences (Isfahan, Iran) approved the study protocol, and each subject signed an informed consent form prior to the study commencement. This study has been registered at the Iranian Registry of Clinical Trials (www.IRCT.IR) under registration number IRCT201206249662N4.

Study inclusion criteria were body mass index (BMI) $\leq 30$, systolic blood pressure (SBP) $> 140$ mmHg and/or diastolic blood pressure (DBP) $> 90$ mmHg. Exclusion criteria were type 1 or 2 diabetes, chronic pancreatitis, liver cirrhosis, kidney stones, renal failure, use of non-steroidal anti-inflammatory drugs, use of antioxidant or vitamin supplements, intense physical activity ($>$ 5 h/week), smoking habit, being vegetarian or having any restrictive dietary requirements and pregnancy.

**Study design.** The present study was designed as a single-blind clinical trial. Included subjects ($n = 21$) were assigned to receive either natural PJ (*Punica granatum* cv. 'shirin') (150 ml/day) ($n = 11$) between lunch and dinner or 150 ml water (as placebo) at the same time ($n = 10$). Patients were alternatively assigned to PJ or control group. The treating physician and laboratory staff remained blind as to the intervention type throughout the study.

**Test drinks and total anthocyanin determination.** Pomegranates were handpicked, washed and the entire fruit then squeezed in a manual juicer. Determination of total anthocyanins in PJ was performed using a pH differential method, as described previously (Lee *et al*., 2005). The method is based on the structural transformations and thereby change in the absorbance of anthocyanins as a function of pH. At pH 1, the colored oxonium form is the predominant form of anthocyanins, while at pH 4.5, anthocyanins turn into colorless hemiketal form. To assay anthocyanins, separate aliquots of PJ were adjusted to pH 1 and pH 4.5 using potassium chloride and sodium acetate buffers, respectively. Following equilibrium, the absorbance of dilutions was read at 515 and 700 nm. The difference in the absorbance of sample is proportional to the anthocyanin content. The absorbance of diluted samples is calculated as follows:

$$A = \left[A(515\,nm)_{pH\ 1.0} - A\ (700\,nm)_{pH\ 1.0}\right] - \left[A(700\,nm)_{pH\ 4.5} - A(700\,nm)_{pH\ 4.5}\right]$$

Knowing the absorbance, the concentration of anthocyanins is calculated using the following equation:

$$Total\ anthocyanin\ content\ (mg/100\ mL) = (A \times MW \times DF)/(\varepsilon \times L \times V)$$

Where MW is the molecular weight (449.2 g $mol^{-1}$), ε is the molar extinction coefficient (26.900 l $cm^{-1}$ $mol^{-1}$), DF is the dilution factor (2), l is the path length (1 cm) and V is the initial sample volume (2 ml). Total anthocyanin content was expressed as mg of cyanidin-3-O-glucoside per 100 ml.

**Anthropometric measurements.** Height was measured to the nearest 0.1 cm with a meter, with participants being barefoot. Body weight was measured to the nearest 0.05 kg using a calibrated digital scales (AMZ 14; Mercury, Tokyo, Japan). The participants were wearing light clothing and no footwear. BMI was calculated as weight in kilograms divided by height in meters squared ($m^2$).

**BP measurement.** BP was measured at baseline and at the end of trial according to a standard protocol. BP recordings were performed after a rest, in the seated state and using a stethoscope and a standard mercury sphygmomanometer. The SBP was defined as the appearance of the first sound (Korotkoff phase 1) and the DBP was defined as the disappearance of the sound (Korotkoff phase 5) during deflating of the cuff.

**FMD measurement.** Endothelium-dependent FMD of the right brachial artery was measured at baseline as well as at the end of trial under conditions described previously (Dickinson *et al.*, 2009). FMD was measured following a 5-min rest and by means of a GE vivid 3 Ultrasound apparatus (AtCor Medical, Solingen, Germany). A BP cuff was inflated around forearm to 200 mmHg for 5 min. Images were recorded at baseline (before inflation), 30 s before cuff release and then every 15 s after cuff release for 3 min. Arterial diameter was measured at the end of end-diastolic phase, coinciding with R-wave on the electrocardiogram. Brachial FMD was calculated as the percentage of change from the baseline diameter. In order to avoid any performance bias, the operator who conducted FMD measurements was kept unaware of the allocation type at the time of the test (Corretti *et al.*, 2002).

**Venous blood collection.** Overnight fasted blood samples (5 ml) were collected from the left antecubital vein between 8:00 and 9:30 am. Blood was taken into vacutainer tubes without anti-coagulant, under quality control and safety procedure. Serum was separated from blood 2–3 h after sampling by centrifugation at 3500–4000 rpm for 10 min. Collected sera were stored at −80 °C until analysis.

**Biochemical analysis.** Serum lipid profile [comprising total cholesterol, triglycerides, low- (LDL-C) and high-density lipoprotein cholesterol (HDL-C)] along with high-sensitivity C-reactive protein (hs-CRP) and fasting blood sugar (FBS) were determined using an automated enzymatic assay (Pars Azmoon, Tehran, Iran) on a Hitachi 902 autoanalyzer. Serum concentration of adhesion molecules including intracellular adhesion molecule-1 (ICAM-1), vascular endothelial adhesion molecule 1 (VCAM-1) and E-selectin together with interleukin-6 (IL-6) were measured by an enzyme-linked immunosorbent assay method with commercial kits (Boster Biological Technolog, Wuhun, China). Apo B and Apo A were quantified using a modified commercially available immunoturbidimetric assay according to the kit instructions (Pars Azmoon, Iran, Tehran).

**Statistical analysis.** All statistical analyses were performed using SPSS software package for Windows. Data were expressed as means ± SD. Between-group comparisons were made using independent samples *t*-test (for normally distributed data) or Mann–Whitney U test (for non-normally distributed data). Within-group comparisons were performed using paired *t*-test (for normally distributed data) or Wilcoxon signed-ranks test (for non-normally distributed data). Bivariate correlations between changes in SBP, DBP and FMD, and anthropometric and biochemical parameters were evaluated using Pearson's (in case of normally distributed data) and Spearman's (in case of non-normally distributed data) correlation coefficient. In addition, stepwise multiple linear regression analysis was performed to identify the parameters that could predict the changes in SBP, DBP and FMD, as primary outcome measures (dependent variables). Dependent variables that entered into the model included changes in ICAM-1, VCAM-1, E-selectin, hs-CRP, IL-6, total cholesterol, LDL-C, HDL-C, triglycerides, apo A, apo B and FBS. A $p$-value of $< 0.05$ was considered as statistically significant.

## RESULTS

### Demographic findings

Baseline demographic biochemical parameters of PJ and control groups are summarized in Table 1. The groups were comparable regarding age, gender, weight, height, waist circumference, BMI and FMD, as well as serum concentrations of hs-CRP, IL-6, ICAM-1, VCAM-1, total cholesterol, LDL-C, HDL-C and triglycerides ($p > 0.05$). Likewise, no significant difference was observed between the study groups regarding their mean SBP and DBP values at baseline ($p > 0.05$). However, serum E-selectin ($p < 0.001$) and apo B ($p = 0.033$) concentrations were significantly higher in the PJ compared to control group.

### Total anthocyanin content

Analysis of PJ indicated a total anthocyanin content of 5.8 mg per 100 ml of the administered juice.

### Effect of PJ on blood pressure and FMD

PJ consumption was found to be associated with significant reductions in mean systolic ($p = 0.002$) and DBPs ($p = 0.038$) compared to the control group. However, no significant difference in the extent of FMD changes was found between the groups ($p > 0.05$). Within- and between-group changes in SBP, DBP and FMD during the course of trial are summarized in Tables 2 and 3, respectively.

### Effect of PJ on serum lipoprotein and apolipoprotein concentrations

Magnitude of changes in serum lipid profile parameters (comprising total cholesterol, LDL-C, HDL-C and triglycerides), apo A and apo B was comparable between the PJ and control groups ($p > 0.05$) (Tables 2 and 3).

### Effect of PJ on endothelial function and inflammatory biomarkers

Consumption of PJ was associated with a significant reduction in serum VCAM-1 concentrations ($p = 0.008$). In contrast, serum levels of E-selectin were elevated by the end of trial in the PJ group ($p = 0.039$). Differentials for other parameters (ICAM-1, hs-CRP and IL-6) were not found to be significantly different between the study groups ($p > 0.05$) (Tables 2 and 3).

### Correlation and regression analyses

In bivariate analyses, no significant correlation was found between changes in SBP and any of the assessed parameters nor was any association for DBP and FMD in the PJ group. In the control group, there was no significant change over the study period, neither in SBP nor in DBP. However, significant correlations were found between changes in FMD and changes in the

**Table 1. Baseline characteristics of the study groups**

| *p*-value | Control group (*n* = 10) | Pomegranate juice group (*n* = 11) | |
|---|---|---|---|
| > 0.05 | 46.90 ± 12.36 | 58.91 ± 5.06 | **Age (years)** |
| > 0.05 | 70.0 | 72.7 | **Female (%)** |
| > 0.05 | 73.50 ± 5.98 | 68.00 ± 10.05 | **weight (kg)** |
| > 0.05 | 161.70 ± 9.46 | 159.18 ± 6.03 | **Height (cm)** |
| > 0.05 | 100.20 ± 9.89 | 97.27 ± 9.45 | **Waist circumference (cm)** |
| > 0.05 | 27.95 ± 4.14 | 26.79-3.47 | **BMI (kg/m$^2$)** |
| > 0.05 | 128.00 ± 13.17 | 130.91 ± 13.00 | **SBP (mmHg)** |
| > 0.05 | 85.00 ± 8.50 | 80.00 ± 8.94 | **DBP (mmHg)** |
| > 0.05 | 0.27 ± 0.05 | 0.24 ± 0.09 | **FMD (%)** |
| > 0.05 | 0.96 ± 0.54 | 1.82 ± 1.58 | **Hs-CRP (ng/ml)** |
| > 0.05 | 662.50 ± 175.59 | 577.36 ± 171.39 | **ICAM-1 (ng/ml)** |
| > 0.05 | 517.60 ± 144.95 | 392.55 ± 164.46 | **VCAM-1 (ng/ml)** |
| < 0.001 | 36.27 ± 37.61 | 122.60 ± 20.23 | **E-Selectin (ng/ml)** |
| > 0.05 | 6.65 ± 3.77 | 7.49 ± 4.75 | **IL-6 (ng/ml)** |
| > 0.05 | 118.10 ± 14.17 | 12.05 ± 14.75 | **Apo A (mg/dl)** |
| 0.033 | 73.90 ± 12.54 | 87.39 ± 14.15 | **Apo B (mg/dl)** |
| > 0.05 | 187.40 ± 31.52 | 208.91 ± 38.21 | **TC (mg/dl)** |
| > 0.05 | 39.70 ± 6.55 | 48.73 ± 7.51 | **HDL-C (mg/dl)** |
| > 0.05 | 161.40 ± 125.60 | 149.09 ± 50.44 | **Triglycerides (mg/dl)** |
| > 0.05 | 109.00 ± 26.80 | 125.09 ± 24.94 | **LDL-C (mg/dl)** |
| > 0.05 | 87.80 ± 10.91 | 90.09 ± 6.11 | **FBS (mg/dl)** |

Values are expressed as mean ± SD or %. BMI: body mass index; Hs-CRP: high-sensitivity C-reactive protein; ICAM-1: intracellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1; IL-6: interleukin-6; apo A: apolipoprotein A; apo B: apolipoprotein B; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FBS: fasting blood sugar.

**Table 2. Pre- vs. post-trial values of the evaluated parameters in the study groups**

| | Pomegranate juice group (*n* = 11) | | | Control group (*n* = 10) | | |
|---|---|---|---|---|---|---|
| | **Baseline** | **Post-trial** | ***p*-value** | **Baseline** | **Post-trial** | ***p*-value** |
| **FMD (%)** | 0.23 ± 0.09 | 0.29 ± 0.07 | 0.034 | 0.27 ± 0.04 | 0.28 ± 0.07 | 0.705 |
| **SBP (mmHg)** | 130.91 ± 13.00 | 124.55 ± 15.72 | 0.008 | 128.00 ± 13.17 | 128.00 ± 13.17 | 1.00 |
| **DBP (mmHg)** | 80.00 ± 8.94 | 76.36 ± 6.74 | 0.046 | 85.00 ± 8.50 | 85.00 ± 8.05 | 1.00 |
| **Hs-CRP (ng/ml)** | 1.81 ± 1.57 | 1.49 ± 2.28 | 0.050 | 91.00 ± 8.75 | 91.00 ± 8.75 | 0.269 |
| **ICAM-1 (ng/ml)** | 577.36 ± 171.39 | 524.36 ± 208.79 | 0.028 | 662.50 ± 177.58 | 654.20 ± 177.68 | 0.066 |
| **VCAM-1 (ng/ml)** | 392.55 ± 164.46 | 233.09 ± 107.87 | 0.008 | 517.60 ± 144.95 | 516.40 ± 148.76 | 0.635 |
| **E-Selectin (ng/ml)** | 122.59 ± 20.23 | 152.33 ± 33.69 | 0.050 | 36.27 ± 37.61 | 39.54 ± 45.70 | 0.109 |
| **IL-6 (ng/ml)** | 7.49 ± 4.75 | 4.38 ± 1.04 | 0.086 | 6.65 ± 3.76 | 6.01 ± 3.56 | 0.655 |
| **Apo A (mg/dl)** | 120.50 ± 14.74 | 122.63 ± 19.90 | 0.563 | 118.10 ± 14.16 | 118.700 ± 17.66 | 0.234 |
| **Apo B (mg/dl)** | 87.39 ± 14.15 | 93.18 ± 25.98 | 0.756 | 73.90 ± 12.53 | 72.30 ± 12.74 | 0.175 |
| **TC (mg/dl)** | 208.91 ± 38.20 | 218.73 ± 42.81 | 0.109 | 187.40 ± 31.51 | 187.00 ± 30.27 | 0.276 |
| **HDL-C (mg/dl)** | 48.73 ± 7.51 | 49.27 ± 8.06 | 0.507 | 39.70 ± 6.55 | 40.40 ± 6.91 | 0.276 |
| **Triglycerides (mg/dl)** | 149.09 ± 50.44 | 171.18 ± 78.92 | 0.401 | 161.40 ± 125.60 | 165.60 ± 124.32 | 0.141 |
| **LDL-C (mg/dl)** | 125.09 ± 24.94 | 127.27 ± 24.22 | 1.000 | 109.00 ± 26.80 | 109.40 ± 25.82 | 0.334 |
| **FBS (mg/dl)** | 90.09 ± 6.10 | 90.64 ± 7.004 | 0.528 | 87.80 ± 10.91 | 89.10 ± 11.34 | 0.240 |

Values are expressed as mean ± SD or %. BMI: body mass index; Hs-CRP: high-sensitivity C-reactive protein; ICAM-1: intracellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1; IL-6: interleukin-6; apo A: apolipoprotein A; apo B: apolipoprotein B; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FBS: fasting blood sugar.

following parameters: ICAM-1 ($r = -0.649$; $p = 0.042$), E-selectin ($r = 0.702$; $p = 0.024$), apo A ($r = -0.679$; $p = 0.031$), total cholesterol ($r = 0.877$; $p = 0.001$) and IL-6 ($r = -0.784$; $p = 0.013$). In stepwise linear regression analysis, changes in SBP, DBP and FMD were separately entered into the model as dependent variables. Independent variables included changes in ICAM-1, VCAM-1, E-selectin, hs-CRP, IL-6, total cholesterol, LDL-C, HDL-C, triglycerides, apo A, apo B and FBS. Linear regression findings indicated that none of the entered independent variables could predict changes in SBP, DBP or FMD. However, in the control group, changes in serum total cholesterol [OR: 0.719; 95% CI: 0.023–0.061); $p = 0.001$] and apo A [OR: −0.384; 95% CI: −0.012–0.001); $p = 0.026$] were found as significant confounders of FMD changes during the course of trial.

**Table 3. Comparison of the magnitude of changes in the evaluated parameters between the study groups**

| Groups | PJ group | 95% CI | Control group | 95% CI | *p*-value |
|---|---|---|---|---|---|
| FMD (%) | 0.05 ± 0.07 | 0.008 – 0.10 | 0.01 ± 0.09 | −0.05 – 0.07 | 0.208 |
| SBP (mmHg) | −6.36 ± 5.05 | −9.75 – −2.97 | 0.00 ± 0.00 | - | 0.002 |
| DBP (mmHg) | −3.64 ± 5.05 | −7.03 – −0.25 | 0.00 ± 0.00 | - | 0.038 |
| Hs-CRP (ng/ml) | −0.33 ± 0.89 | −0.93 – 0.27 | 0.17 ± 0.42 | −0.13 – 0.47 | 0.124 |
| ICAM-1 (ng/ml) | −53.00 ± 79.72 | −106.56 – 0.56 | −8.30 ± 15.00 | −19.03 – 2.43 | 0.096 |
| VCAM-1 (ng/ml) | −159.40 ± 158.86 | −266.18 – −52.73 | −1.20 ± 7.73 | −6.73 – 4.33 | 0.008 |
| E-Selectin (ng/ml) | 29.74 ± 36.53 | 5.20 – 54.28 | 3.27 ± 8.36 | | 0.039 |
| IL-6 (ng/ml) | −3.11 ± 4.82 | −6.35 – 0.13 | −0.63 ± 2.36 | −2.32 – 1.06 | 0.387 |
| Apo A (mg/dl) | 2.14 ± 18.65 | −10.39 – 14.66 | 0.60 ± 4.97 | −2.96 – 4.16 | 0.797 |
| APO B (mg/dl) | 5.80 ± 24.70 | −10.80 – 22.38 | −1.60 ± 3.57 | −4.15 – 0.95 | 0.349 |
| TC (mg/dl) | 9.82 ± 32.82 | −12.23 – 31.87 | −0.40 ± 1.51 | −1.48 – 0.68 | 0.327 |
| HDL-C (mg/dl) | 0.55 ± 2.58 | −1.19 – 2.28 | 0.70 ± 1.77 | −0.56 – 1.96 | 0.863 |
| TG (mg/dl) | 22.09 ± 58.13 | −16.96 – 61.14 | 4.20 ± 10.60 | −3.38 – 11.78 | 0.338 |
| LDL-C (mg/dl) | 2.18 ± 23.36 | −13.51 – 17.88 | 0.40 ± 1.78 | −0.87 – 1.67 | 0.806 |
| FBS mg/dl | 0.55 ± 3.30 | −1.67 – 2.76 | 1.30 ± 1.34 | 0.34 – 2.26 | 0.509 |

Values are expressed as mean ± SD plus 95% confidence interval or %. BMI: body mass index; Hs-CRP: high-sensitivity C-reactive protein; ICAM-1: intracellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1; IL-6: interleukin-6; apo A: apolipoprotein A; apo B: apolipoprotein B; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FBS: fasting blood sugar.

## DISCUSSION

The results of this study demonstrated that consumption of PJ is associated with significant hypotensive effects. In addition, interesting reduction of serum VCAM-1 was observed, suggesting potential efficacy of PJ against endothelial dysfunction and vascular inflammation. Nonetheless, FMD was not significantly altered by PJ despite promising within-group improvement. A previous trial among hypertensive adults documented a dose–response relationship between increased fruit and vegetable consumption and improved FMD (Castilla *et al.*, 2008). Recent studies have investigated the beneficial effects of anthocyanin-rich fruit juices such as cranberry and blood orange juice in subjects with coronary artery disease. Although such products could reduce carotid femoral pulse wave velocity and mitigate arterial stiffness, they had no effect on brachial artery FMD (Dohadwala *et al.*, 2011; George *et al.*, 2009; Morand *et al.*, 2011). Since a marked reduction of FMD was observed in the PJ but not control group, PJ appears to be more efficacious in the improvement of endothelial function compared with the above mentioned beverages. PJ has a high content of anthocyanins, vitamin C and carotenoids, which are all micronutrients known to exert cardioprotective and endothelial ameliorating effects (George *et al.*, 2009). Serum elevation of E-selectin by PJ was a somewhat contradictory finding as there was a decreasing trend for other evaluated CAMs following PJ supplementation. This finding could be partially attributed to the higher baseline concentrations of E-selectin in the PJ compared to control group, resulting in a greater effect size and increased likelihood of detecting a significant difference in the PJ group.

The principal finding of the present study is that 2-weeks consumption of PJ significantly decreases DBP and SBP in hypertensive patients. This is consistent with the findings of a previous study in daily consumption of 50 ml of PJ for two weeks was reported to cause a 5% decline in SBP (Aviram and Dornfeld, 2001). In this latter study, a marked reduction in serum angiotensin-converting enzyme activity (36%) was also observed which justifies the observed hypotensive effects. In another trial by Aviram *et al.* (2004), consumption of PJ for one year was found to be associated with about 21% reduction in SBP and significant decreases in carotid intima-media thickness, LDL-oxidation, anti-oxidized LDL antibody titers. Furthermore, serum paraoxonase-1 activity, along with total antioxidant status, was augmented by PJ consumption. Similar to the present results, no effect on lipid profile parameters as well as apo A and apo B levels was observed. In addition, DBP values remained unaltered by the end of trial. This discrepancy in DBP changes could be attributed to the basic difference in the study populations as participants of the Aviram *et al.* (2004) study were patients with severe carotid artery stenosis whereas the present study recruited hypertensive patients. Further to the BP lowering activity, PJ has been shown to possess considerable anti-atherosclerotic antioxidant and anti-inflammatory effects in both human subjects and mouse models (Stowe, 2011).

Hs-CRP is a sensitive marker of vascular inflammation and an emerging risk factor for CVD (Libby, 2006). Although between-group comparisons implied a decreasing trend in serum hs-CRP following PJ consumption, no significant effect was observed in the between-group analysis. The most plausible explanation for not detecting any significant difference between the study groups in terms of hs-CRP and FMD is the limited number of subjects which makes the study underpowered for these parameters. As another finding, PJ exerted favorable effects on the circulating levels of endothelial adhesion molecules. This is an interesting finding as upregulation of endothelial adhesion molecules, including E-selectin, ICAM-1 and VCAM-1, play a crucial role in the earliest phases of atherosclerosis (Blankenberg *et al.*, 2003; Gonzalez and Selwyn, 2003).

Unlike the aforementioned parameters, no significant alteration was found in the serum levels of IL-6, apo A, apo B and lipid profile parameters. While the possibility of a lack of effectiveness on these indices is not excluded, a definitive judgment requires studies with

longer supplementation period and higher intakes of PJ. Heretofore, a number of bioactive molecules with anti-inflammatory effects have been identified in pomegranate. These phytochemicals, e.g. punicalagin, punicalin, strictinin A and granatin B, have been shown to reduce the generation of nitric oxide and PGE2 by inhibiting the expression of pro-inflammatory proteins (Lee *et al*., 2008; Romier *et al*., 2008). Overall, the most frequent and bioactive class of compounds in PJ appear are polyphenols. PJ contains higher levels of polyphenolic compounds than other fruit juices, such as grapefruit, orange, apple and cranberry juice (Basu and Penugonda, 2009). The major polyphenols in the PJ include ellagitannins and anthocyanins, which are believed to contribute to cardiovascular health via a wide range of activities, e.g. antioxidant, anti-inflammatory and immunomodulatory actions (Gil *et al*., 2000; Lansky and Newman, 2007).

### Limitations

The present study was limited in a number of ways: First, the patients were not blinded with respect to the intervention. This is in part due to the distinct taste and appearance of PJ which makes the preparation of a matched placebo treatment a very hard task. Second, the alternative assignment of participants to PJ and control groups hindered concealment of allocations from the administering physician. Third, no food frequency questionnaire was used nor daily intake of fruits and vegetables was monitored in the present trial. Hence, it remains unclear that group differences in the intake of micro- and macronutrients had any significant effect on the alterations observed in the evaluated parameters. Fourth, PJ in the present trial was characterized as to a single class of phytochemicals namely anthocyanins. It is suggested that future studies provide a more comprehensive phytochemical analysis, characterize PJ with respect to other phytochemical subsets (e.g. total phenolics, flavonoids, tannins, carotenoids, *n*-3 polyunsaturated fatty acids etc) and standardize the administered dosage based on the total antioxidant intake. Finally, the study population size was relatively few due to the pilot design, and this might have negatively influenced the power to detect significant differences in a number of parameters. Therefore, future randomized double-blind trials are warranted to confirm the preliminary findings found in this pilot trial in larger populations and over a longer period of time. The impact of PJ consumption on FMD – as the main outcome measure for the assessment of endothelial function – is of particular interest as there was trend toward improvement of FMD in the present study but this did not reach statistical significance, probably due to the study being underpowered for this outcome. Testing the effect of consuming different PJ volumes is also greatly recommended to determine if larger volumes of intake are associated with enhanced hypotensive and anti-inflammatory effects.

## CONCLUSION

In summary, findings from the present pilot trial provided evidence for the beneficial impact of PJ consumption on both systolic and DBPs in hypertensive individuals. Besides, consumption of PJ was found to be associated with significant reductions in serum VCAM-1 which is a biomarker of endothelial function and vascular inflammation. In light of these promising findings, PJ may be considered as an effective adjunct to the anti-hypertensive medications and also as a constituent of daily regimen for patients who are high risk for hypertension and CVD. However, any definitive judgment on the efficacy of PJ remains to be made in light of the findings of future larger scale and double-blind trials.

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### Conflict of Interest

The authors have declare that there is no conflict of interest.

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